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MESENCHYMAL STEM CELLS MIGRATE INTO OSTEOARTHRITIS JOINT FROM SYSTEMIC CIRCULATION: AN ANIMAL MODEL

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Purpose: There are not clear evidences about the migration of mesenchymal stem cells from a systemic circulation to another niche as an injury place in the organism. The current study treats to answer this question.

Methods: Mesenchymal stem cells (MSCs) have been injected via intravenous and directly into the monkey joint, these MSCs were previously characterized by flow cytometry revealing that were positive for CD90 and CD44 and negatives for CD34 and CD45 indicating that presence of MSCs markers. CD105 enriched sub-population of MSCs was labelled with oxacarbocyanine – DiO (green) when they were injected via intravenous (IV), with octadecyl (C18) indocarbocyanine – DiI (red) when they were injected directly into the knee, intra-articular (IA) and another group of MSCs were modified to express constitutively the green fluorescence protein (GFP) to follow their evolutions through the animals injected by different ways and their localization. The animal models used were adult monkeys *Macaca Fascicularis* which had been injured into the left knee to create an Osteoarthritis (OA) animal model and the right knee was used as control. First step: one group of three animals was injected with 1×10^6GFP-MSCs/KG of animal in the vein twice and these animals were sacrificed only one week after the second injection because the purpose of this experiment was following the GFP-MSCs into the organism. Second steep: one group of two animals was injected twice in the vein with 1×10^6 CD105$^+$-MSCs labelled with DiO only and another two animals were injected directly in the knee twice with 1×10^6 CD105$^+$-MSCs labelled with DiI at the same time these animals were injected twice in the vein with 1×10^6CD105$^+$-MSCs labelled with DiO. The injections were realized into the animals with an interval of one week between them. The animals were sacrificed one month after treatment.

Results: Immunohistochemistry analysis of the tissues from the animals revealed that and GFP-MSCs and CD105$^+$-MSCs migrated towards the injured knee joint. Neither labelled cells nor teratomes were found in other vital organs into the animals. The CD105$^+$-MSCs labelled with DiI (red) and with DiO (green) were only located in the injured knee (0.01% from the total injected) and they were not found in the normal one. Furthermore CD105$^+$-MSCs labelled with DiI (red) and with DIO (green) were found in crossed ligaments and knee muscle (vastus medialis), synovial membrane, both meniscus and cartilage of patello femoral groove all of them only in the injured joint and not in the normal one. An exhaustive immunofluorescence analysis of DiO labelled cells founded in the injured knee revealed that they were negatives for CD68, which is a macrophage antibody marker, and 4% of CD105$^+$-MSCs labelled with DiO (green) were positives for SDF-1, which is a marker of osteoclastogenesis and produces liberation of IL-6, in the patello femoral groove and crossed ligaments of injured joint and not in the normal one.

Conclusions: These results seem indicate that:

1. The MSCs injected in a peripherical vein go to injured tissue, in this case injury knee.
2. The MSCs injected directly in an injured place keep in the same place.
3. The MSCs could be recruitment by SDF-1.

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SHORT TERM HIGH-FAT DIET INDUCES OSTEOPHYTE FORMATION, APOPTOSIS AND DEGENERATION OF ARTICULAR CHONDROCYTE IN MURINE KNEE JOINT

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Purpose: Obesity and high body mass index are associated with a higher incidence risk of OA. To elucidate the relationship between obesity and osteoarthritis, several attemps have been made to observe the effect of high fat diet (HFD) on murine articular degeneration. Although HFD have succeeded in inducing osteoarthritis after long term treatment (for more than 6months), initial response of articular tissues to HFD is still unknown. The aim of this study is to evaluate sub-acute effect of HFD on histological features of murine knee joint.

Materials and Methods: C57BL/6J male mice were fed a diet containing 32% fat for HFD group and normal diet for control group from 7 weeks of age for less than 12 weeks. Mice feeding a high fat diet and control have been divided into three groups, such as 4 weeks, 8 weeks and 12 weeks of treatment. Whole knee joints were removed by dissection, fixed in 4% paraformaldehyde, and decalcified in EDTA. After dehydration and paraffin embedding, serial 5-μm sagittal sections were made from the whole medial compartment of the knee joint. Three sections were picked up at 100-μm intervals (from lane 1; medial to lane 3; lateral) from the weight-bearing region of each medial plateau of tibia, and were stained with Safranin O-fast green and HE. OA severity in the tibial plateau was evaluated according to Mankin's histologic grading system. Osteophyte area was quantified in each slide using Image-Pro Plus 4.1 software. ColX expression was examined by immunohistochemistry. TUNEL assay was performed and the number of TUNEL positive cells were compared between HFD group and control.

Results: Body weight was increased 10% in 4weeks, 20% in 8 weeks, 50% in 12 weeks of HFD group compared to controls respectively. As soon as 4weeks of HFD treatment, the area of intrapatellar fat pad (IPFP) was increased in HFD group prior to cartilage degeneration. Infiltration of inflammatory cells was observed at synovium and IPFP. Prior to osteophyte formation, increased synovial cells were abundantly observed at anterior edge of tibial plateau. Simultaneously, angiogenesis was activated in IPFP. At 8 weeks of HFD treatment, TUNEL positive cells were abundantly observed at the site of osteophyte formation and superficial or middle zone of articular cartilage. The number of TUNEL positive cells was significantly elevated in HFD group. Osteophyte formation was detected at the medio-anterior edge of tibial plateau. Mankin score was significantly elevated in HFD group from this period. At 12 weeks of treatment, osteophyte formation was apparently advanced in HFD group as a result of activated endochondral ossification. Type X collagen expression in articular cartilage was enhanced at weight bearing region in HFD group.

Conclusion: We demonstrated that osteoarthritic change, such as osteophyte formation, apoptosis and degeneration of articular chondrocyte, could be induced by HFD within three month of treatment. Prior to OA progression, thickening of IPFP accompanied by mild inflammation was observed, suggesting involvement of IPFP in the initiation of OA change. In vivo model described herein offers opportunities to noninvasively induce osteoarthritis without surgery.

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ANTI-INFLAMMATORY EFFECT OF ROSUVASTATIN AND ROSIGLITAZONE SUPPRESSES DEVELOPMENT OF DIET-INDUCED OSTEOARTHRITIS IN HUMAN CRP TRANSGENIC MICE

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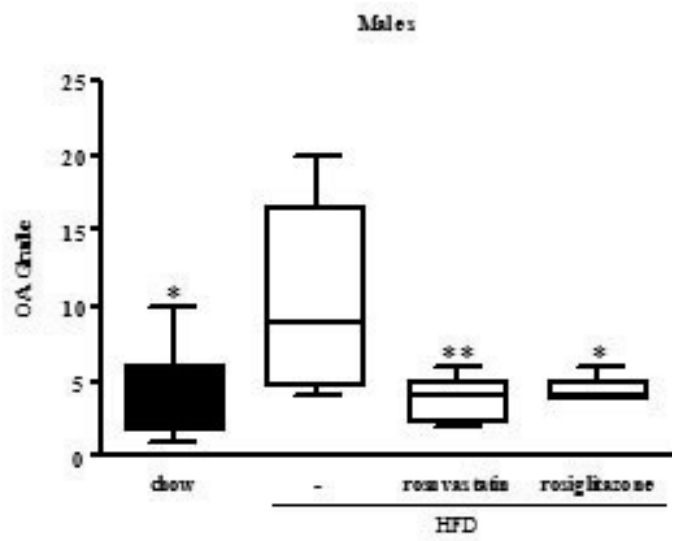
Purpose: Obesity is associated with systemic inflammation and a risk factor for osteoarthritis (OA) development. We hypothesized that metabolic stress-induced inflammation is responsible for the development of high fat diet (HFD)-induced OA in mice and not mechanical overload.

Methods: To test this hypothesis, human C-reactive protein (huCRP) transgenic mice received HFD without or with 0.005% w/w rosuvastatin or 0.018% w/w rosiglitazone, two different types of drugs with anti-inflammatory capacities. Control mice fed chow were also included. After 42 weeks mice were sacrificed and histological grading of the knees was performed to assess OA development. To monitor the overall inflammatory state, systemic huCRP levels were determined. In addition, the adipokines leptin and resistin, for which a role in OA has been suggested, were measured.

Results: Male mice on HFD had significantly higher OA grades than mice on chow and showed no correlation between severity of OA and body weight. HFD-induced OA was significantly inhibited by rosuvastatin or rosiglitazone to OA grades observed in age-matched control mice on

chow. Both treatments resulted in reduced huCRP levels, indicative for their anti-inflammatory action. Furthermore, a positive correlation was found between the relative individual induction of huCRP evoked by HFD at day 3 and end point OA grade. Leptin and resistin levels did not correlate with OA severity.



Conclusions: HFD-induced OA in mice is due to low grade inflammation and not the consequence of mechanical overload, since no relation between body weight and OA grade was observed. Moreover, the OA process was inhibited to a great extent by treatment with two drugs with anti-inflammatory properties. The inflammatory response to a metabolic high fat challenge may predict the individual susceptibility to develop OA later in life. The use of statins or PPARγ-agonists (such as rosiglitazone) could be a strategy to interfere in the progression of OA.

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EFFECT OF THE SELECTIVE CATHEPSIN K INHIBITOR, MIV-711, ON MARKERS OF CARTILAGE DEGRADATION AND ON GROSS AND HISTOPATHOLOGY SCORES IN DOGS SUBJECTED TO PARTIAL MEDIAL MENISCECTOMY, AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS

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Purpose: Cathepsin K is a cysteine protease capable of degrading several bone and cartilage matrix components. Previously, a pan-cathepsin inhibitor provided cartilage-protective effects in dogs subjected to partial medial meniscectomy, an experimental model of osteoarthritis (OA). In the current study we evaluated MIV-711, a potent (Ki: 0.98 nM) and highly selective cathepsin K inhibitor (>1,000-fold selectivity vs. related cathepsin enzymes) in the same model.
Methods: Female beagle dogs were subjected to partial medial meniscectomy of the left knee and were treated with either vehicle (n = 15) or MIV-711 (n = 15) at 30 μmol/kg (17 mg/kg) once daily starting the day before surgery. Treatment continued for 28 days after surgery followed by necropsy on day 29. Plasma, synovial fluid and urinary samples were collected at various time points during the experiment and at necropsy. Biomarkers of collagen degradation were measured using commercially available ELISA kits. Gross and microscopic changes of the femorotibial joints were assessed.
Results: MIV-711 significantly reduced CTX-II levels (marker of cartilage degradation) in urine (by 80%, $p < 0.001$) compared to baseline and by 61% ($p < 0.001$) compared to vehicle. Synovial fluid levels of CTX-II in both knees were also significantly reduced by 55–57%, ($p < 0.001$) compared to baseline and by 31–36% ($p < 0.01$) compared to vehicle. Synovial levels of MIV-711 were significantly higher in the operated left knee than in the right knee. Subjective and calculated gross pathology scores were reduced by 25–37% in the femur and in the tibia by 13–37%in MIV-711-treated animals compared to vehicle. Total lesion area in the tibia was 26% lower in MIV-711-treated animals and the most severe lesion area was reduced by 75%. Various scores of tibia cartilage histopathology were 9–17% lower in the tibia of MIV-treated dogs.
Conclusions: We have previously shown that once daily oral dosing of MIV-711 in dogs subjected to partial medial meniscectomy had a positive effect on markers of bone resorption while maintaining markers of bone formation. The present results now show that selective cathepsin K inhibition achieved by once daily oral dosing with MIV-711 affects markers of cartilage degeneration in a dog OA model. Taken together, these data suggest MIV-711 may be beneficial not only for treatment of osteoporosis but also for OA.

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ESTABLISHING THE HOMOZYGOUS SPONDYLOEPIPHESEAL DYSPLASIA CONGENITA MUTATION AS A MODEL FOR OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is one of the leading causes of joint pain and disability in the United States. There is a lack of effective treatments for the disease because of a poor understanding of its pathogenesis. Murine models, such as spondyloepiphyseal dysplasia congenita (sedc), are important in further understanding OA. The purposes of the present study are to (1) establish the homozygous sedc mutation (s/s) as a model of osteoarthritis; (2) characterize the articular cartilage in sedc mice by identifying cellular changes over time and localizing the major structural proteins present in the extracellular matrix; (3) to identify a possible mechanism involved in the pathogenesis of OA.
Methods: Knee and temporomandibular joints of homozygous wild-type (+/+) and homozygous sedc (s/s) mice were compared histologically at 2, 6 and 9 months. The mice were euthanized and their tissues were prepared and stained using Fast Green and Safranin O to show the presence and localization of proteoglycans and collagen. Immunohistochemical staining (IHC) for Ddr2 and Mmp-13 was also performed to detect changes in the presence of both of these degradative proteins. In addition, toluidine blue stained sections were analyzed to determine the matrix vs. chondron area in both the mutant and the wild-type mice. TEM photomicrographs were taken for comparison of cell diameter, pericellular matrix and fibril diameter.
Results: Histological analysis of the mutant showed increased pericellular space and disorganization of the chondrocytes. Rather than superficial fissuring as seen in the sedc heterozygous model, the sedc homozygous mice displayed increased fissuring only along the tidemark that separates calcified from non-calcified cartilage. Homozygous mutant mice displayed increased cartilage separation, increased pericellular diameter, increased proteoglycan staining, and cell clustering as early as 2 months. IHC staining showed a lack of upregulated Ddr2 and Mmp-13 in mutant mice. TEM of the mutant showed atypical cell morphology including cytoplasmic membrane extensions, amorphous material in the pericellular matrix and smaller diameter collagen fibrils in the interterritorial matrix.
Conclusions: Homozygous sedc mice develop premature OA in comparison with wild-type contols. Therefore, the homozygous mutant presents itself a valuable murine model of the study of OA. However, the s/s mouse is unlike other OA models in that the tissue degeneration does not appear to be typical of other models that invoke the enzymatic degredation pathway involving Ddr2, HtrA1 and Mmp-13.

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OSTEOARTHRITIS-LIKE CHANGES IN THE MURINE KNEE JOINT BY MEANS OF FORCED RUNNING

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Purpose: Various murine knee osteoarthritis(OA)models are known. However, few non-invasive model has been developed. In this study, we developed murine knee OA model with forced running on the treadmill and evaluate the OA-like changes by X-ray and histology.
Methods: To 9-week old CB57/BL6 mice, forced running were applied 3 times a week (15minutes a once; running distance=165m). At 2, 4, 6, 8 weeks, the knee joints were excised and morphologically analyzed with magnified X-ray.Further, they were subjected to histological analysis (HE, Safranin-O, Toluidine blue).OARSI score was used to semi-quantitative evaluation.And they were immunostained by typeII, X collagen.